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14. ABSTRACT

The goal was to investigate the potential of resveratrol, genistein and (-)-epigallocatechin-3-gallate (EGCG), alone and in combination, to protect against prostate cancer in a transgenic rat model (TRAMP). The specific aims were 1) to investigate the potential of these polyphenols, alone and in combination, to suppress the development of spontaneously developing prostate tumors and 2) to investigate how these polyphenols regulate sex steroid- and specific growth factor- receptor and ligand expression as mechanism of prostate cancer prevention. Single administration of genistein and resveratrol in the diet, but not EGCG in the water, and combination genistein and resveratrol suppressed the development of spontaneously developing poorly differentiated carcinomas in the prostate. EGCG down-regulated cell proliferation in the VP, but not in the DLP. EGCG increased apoptotic indices in the VP, but not in the DLP. These three polyphenols differentially regulate androgen-, estrogen- and EGF-receptor, the IGF-signaling proteins and the extracellular regulating kinases. The primary target of the polyphenols appears to be the IGF-signaling proteins.

15. SUBJECT TERMS
Genistein, Resveratrol, EGCG, Prostate Cancer Chemoprevention, Mice

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Introduction

Asians consuming a diet high in soy products have reduced incidence of clinically manifested prostate cancers. Likewise, Asians have a long history of drinking tea. Significant components of these two staples of the traditional Asian diet are the polyphenolic compounds. The primary polyphenols associated with prostate chemoprevention are the soy isoflavone, genistein, and the tea catechin, (-)-epigallocatechin-3-gallate (EGCG). Another polyphenol that has recently received attention as a cancer suppressor is resveratrol, a component of grapes. The goal of this research was to investigate the potential of these three pure polyphenols, alone and in combination, to protect against prostate cancer in a model of spontaneously developing prostate cancer model (TRAMP mice). In this manner, it may be possible to ingest moderate amount of each of these foods/chemicals, as opposed to mega amounts of one, and receive an additive or synergistic protective effect without adverse effects with possible elevated exposure.

Body

Aim 1) To investigate the potential of the polyphenols, genistein, EGCG and resveratrol, alone and in combination, to protect against prostate cancer. This has been evaluated in the TRAnsgenic Mouse Prostate adenocarcinoma (TRAMP) model that spontaneously develops prostate cancer.

As previously reported we have demonstrated that genistein suppresses spontaneously developing prostate tumors in rats (1). Recently, we demonstrated that resveratrol, but not EGCG, was also able to suppress spontaneously developing prostate cancer in TRAMP mice. Based on these results and our originally proposed Task 1 where we stated that we would further investigate in combination those nutritional agents that alone demonstrate efficacy, we have investigated the potential of resveratrol and genistein in combination, and not EGCG with genistein or resveratrol, to suppress spontaneously developing prostate tumors. Accordingly, the following groups of animals were bred and treated (Task 1, Phase II): Group 1) AIN-76A diet (Controls); Group 2) 125 mg Genistein + 313 mg Resveratrol/kg AIN-76A diet (L-G+R); Group 3) 250 mg Genistein + 625 mg Resveratrol/kg AIN-76A diet (H-G+R).

Following necropsy of these animals at 30 weeks, the tissues were processed, sectioned and H&E stained. Dr. Isom Eltoum, a Board Certified Pathologist, blindly scored each coded sample using the following grading scale developed specifically for rodents: normal tissue: NT, low-grade PIN: L-PIN, high-grade PIN: H-PIN, well-differentiated carcinoma: WDC, moderately differentiated carcinoma: MDC, or poorly differentiated carcinoma: PDC (3). In this study, the pathologist scored the DLP and VP individually in order for us to gain more information on the origin and progression of the tumors. Using a proportionality score for the DLP and VP, we were able to discern the lesion scores in both of these prostate lobes.

In control mice, we found that the most advanced case of prostate cancer, PDC, occurred to a greater extent in the VP compared to the DLP (15% and 1% respectively) (Table 1). This is in spite of the reports that preneoplastic lesions occur first in the DLP (3). We interpret this to mean that while the first lesions occur in the DLP, the PDC progresses faster in the VP. In regards to the combination treatments, the low dose (L-G+R) did not have a protective effect against PDC in the DLP or VP. On the other hand, the combination high dose (H-G+R) decreased the percent of PDC from 15% to 3% in the VP. Averaging the proportionality scores from the DLP and VP, we calculated that the average overall PDC

score in the TRAMP prostate was 8% and 1.5% in control compared to H-G+R group, with the arrest of PCa occuring primarily in the VP at L-PIN stage.

In our previous reports, we demonstrated that genistein and resveratrol when given alone, resulted in a 2 fold and 7.7 fold decrease in PDC with these nutritional polyphenols, respectively. Since the same doses of combinational genistein and resveratrol diet resulted in a 5 fold decrease in proportionality score, we conclude that these two polyphenols do not provide a synergistic chemopreventive effect, but they do work in combination for chemoprevention.

Aim 2) To investigate the potential of genistein, EGCG and resveratrol to regulate sex steroid- and specific growth factor- receptor and ligand expression as mechanisms of prostate cancer prevention. We have previously reported the effect of the individual polyphenols.

From the prostates of mice exposed ± combination genistein, resveratrol and EGCG, we have recently measured protein expression of the androgen receptor (AR), estrogen receptors (ERs), insulin-like growth factor-I (IGF-I) signaling, and extracellular signaling regulating kinases-1 and 2 (ERK-1 and ERK-2). For these determinations, we used only the original treatment doses: G: 250 mg genistein/kg diet; R: 625 mg resveratrol/kg diet and E: 0.06% EGCG in tap water.

In the dorsolateral prostate, using western blot analysis and ELISA, we found that combinational G+R, but not G+E and R+E, resulted in significantly increased AR protein levels (Figure 1). ER-alpha and ER-beta were not altered by all combination treatments. In regards to the IGF-signaling pathway, all three combinations (G+R, G+E and R+E) down regulated IGF-1 and IGF-1R (Figure 2). Only R+E treatment affected IGF-BP3, resulting in down-regulation of the binding protein. For the ERKs in the DLP, we found that only G+E had an effect on the total ERK-1 and 2, significant up-regulation (Figure 3). Importantly, the treatments did not alter phospho-ERK levels (Figure 4), indicating that there was no increase in activated ERKs. We conclude that the primary means of these polyphenols regulating prostate cancer in the prostate is *via* the IGF-signaling pathway, but we do not discount the role of the other proteins or other unidentified regulators.

In the VP, none of the sex steroid receptors (Figure 5) or the ERKs (total- and phosho-ERKs) (Figures 7 and 8) were differentially regulated by the combination treatments. On the other hand, IGF-1 was significantly down-regulated and IGF-1R was significantly up-regulated by G+E treatment, but not by the other combination treatments (Figure 6).

Statistical analysis of histological specimens used Fisher's exact test to determine significance ($P < 0.05$). Analyses were conducted using Microsoft Office Excel 2003 (Microsoft Corp., Seattle, WA). For the biochemical data, experiments were analyzed using one way analysis of variance (ANOVA), with subsequent multiple comparisons. The p-values associated with the individual comparisons were completed using separate t-tests.

Comprehensive Key Research Accomplishments

- Pure genistein (250 mg/kg diet) and resveratrol (625 mg/kg) in the diet, but not 0.06 % EGCG in the water, suppressed spontaneously developing prostate tumors in TRAMPs.
- A combination of 250 mg genistein/kg diet and 625 mg resveratrol/kg diet suppressed prostate cancer development, while one-half the dose of these two compounds did not.

- Genistein and resveratrol decreased cell proliferation and reduced the ratio of cell proliferation to apoptosis in prostates of TRAMP mice. EGCG down-regulated cell proliferation in the VP, but not in the DLP. EGCG increased apoptotic indices (apoptotic bodies) in the VP, but not in the DLP.
- In the DLP, genistein treatment significantly decreased AR, ER-beta, IGF-1, IGF-1R, IGF-BP3, and EGFR protein expressions. AR, ER-alpha, IGF-1R, total-ERKs 1 & 2 and phospho-ERKs 1 & 2 were not significantly altered in genistein treated mice.
- In the VP, genistein treatment significantly decreased AR and phospho-ERKs 1 & 2, but did not significantly alter the other protein levels.
- In the DLP, resveratrol up-regulated AR, ER-beta, IGF-1R and EGFR while down-regulating IGF-1 and phospho-ERK-1. ER-alpha and phospho-ERK-2 were not regulated.
- In the VP, resveratrol treatment significantly decreased IGF-1R and ERKs 1 & 2, but did not alter the other protein levels.
- In the DLP, EGCG significantly down-regulated IGF-1, while up-regulating AR and EGFR.
- In the VP, EGCG treatment down-regulated AR, IGF-1, IGF-1R and phospho-ERKs 1 & 2.
- In the DLP, combination genistein and resveratrol treatments resulted in up-regulated AR and decreased IGF-1 and IGF-1R, but did not alter protein levels of ER-alpha and beta, IGF-BP3 and total and phospho- ERKs-1 and 2.
- In the VP, combination genistein and resveratrol in the diet did not regulate any of the investigated proteins.
- In the DLP, combination genistein + EGCG treatment significantly down-regulated IGF-1 and IGF-1R and up-regulated total ERK-1 and 2 proteins, but did not alter phospho-ERKs 1 and 2, and the sex steroids.
- In the VP, combination genistein + EGCG treatment significantly down-regulated IGF-1 and up-regulated IGF-1R but did not affect the other protein expression.
- In the DLP, combination resveratrol and EGCG treatment down-regulated IGF-1, IGF-1R and IGF-BP3, but none of the other proteins.
- In the VP, combination resveratrol and EGCG treatment did not alter any of the proteins investigated.

Reportable Outcomes

Manuscripts

Harper, C, Patel, B.B., Wang, J., Eltoum, I.E., Lamartiniere, C.A. Resveratrol Suppresses Spontaneously Developing Prostate Cancer in Transgenic Mice. Submitted to Carcinogenesis, 2007.

Harper, C, Patel, B.B., Wang, J., Eltoum, I.E., Lamartiniere, C.A. EGCG Suppresses High Grade-Prostatic Intraepithelial Neoplasia in TRAMP mice: Mechanisms of Action. Submitted to The Prostate, 2007.

Meeting Abstracts

Lamartiniere, C.A. Invited presentation to AICR/WCRF International Research Conference on Food, Nutrition and Cancer. Genistein Chemoprevention of Breast Cancer: Timing and Mechanisms of Action, Washington, DC, July, 2005.

Lamartiniere, C.A. Molecular and Cellular Pathology Seminar: Dietary Polyphenols Protect Against Mammary and Prostate Cancers. University of Alabama at Birmingham Department of Pathology. September, 2005.

Lamartiniere, C.A. University of Gottingen, Germany Seminar: Dietary Polyphenols Protect Against Mammary and Prostate Cancers. February, 2006.

Lamartiniere, C.A. Humboldt University, Berlin, Germany Seminar: Dietary Polyphenols Protect Against Mammary and Prostate Cancers. February, 2006.

Lamartiniere, C.A. Technische Universitat, Dresden, Germany Seminar: Dietary Polyphenols Protect Against Mammary and Prostate Cancers. February, 2006.

Lamartiniere, C.A. Invited Speaker at Conference on Aging Men in Salzburg Austria: Dietary Polyphenols Protect Against Prostate Cancers. February, 2006.

Harper, C. Wang, J., Patel, B.J. and Lamartiniere, C.A.. Epigallocatechin-3-gallate (EGCG) down-regulates the androgen receptor and the IGF pathway in the prostate of TRAMP mice. Proceedings of the American Association for Cancer Research. 2006.

Leah Cook, Curt Harper, and Coral A. Lamartiniere. Resveratrol in the diet regulates IGF-1 signaling proteins in the prostate of rats. 97th Annual meeting of the American Association for Cancer Research, Washington, D.C. May 2006.

Leah Cook, Curt Harper, and Coral A. Lamartiniere. Mechanisms of Action of Resveratrol in the Rat Ventral Prostate. Proceedings of Sigma Xi 100: IR-05, 2005. Sigma Xi Scientific Research Society Annual Meeting, Seattle, WA. November 2005.

Harper, C.E., Patel, B.B., Wang, J. and Lamartiniere, C.A. Resveratrol Action on Steroid and Growth Factor Signaling in TRAMP Mice. 2005 Society of Toxicology 44th Annual Meeting, New Orleans, LA. Proceedings of the Society of Toxicology (Program P164) 2005.

Harper, C.E., Patel, B.B., Wang, J. and Lamartiniere, C.A. The prostate cancer chemopreventive effects of resveratrol. 2005 American Association of Cancer Research 96th Annual Meeting, Anaheim, CA. Proceedings of the American Association for Cancer Research 46: 1015, 2005.

Harper, C.E., Patel, B.B., Wang, J. and Lamartiniere, C.A. 2005 Gordon Research Conference: Hormone Action in Development & Cancer, South Hadley, MA. The prostate cancer chemopreventive effects of resveratrol.

Harper, C.E., Patel, B.B., Wang, J. and Lamartiniere, C.A. American Association of Cancer Research 97th Annual Meeting, Washington, DC. Epigallocatechin-3-gallate (EGCG) down-regulates the androgen receptor and the IGF pathway in the prostate of TRAMP mice. Platform Presentation. Prevention Research 10: Tea Catechins. 2006

Harper, C.E., Patel, B.B., Wang, J. and Lamartiniere, C.A. Environmental Mutagen Society 37th Annual Meeting, Vancouver, British Columbia, Canada. Prostate Cancer Chemoprevention Using Dietary

Polyphenol Resveratrol. Platform Presentation. The Role of Dietary Supplements and Food in Human Health. 2006.

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Mark Carpenter Ph.D.
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Curt Harper, M.S.
Natalie Durr, B.S.

Conclusions

Single administration of genistein and resveratrol in the diet, but not EGCG in the water, suppressed the development of spontaneously developing poorly differentiated carcinomas in the prostate. Follow up investigation of the two effective chemopreventive polyphenols, showed that in combination they also suppressed prostate cancer, but no greater effect was achieved than each alone.

Genistein and resveratrol decreased cell proliferation and reduced the ratio of cell proliferation to apoptosis in prostates of TRAMP mice. EGCG down regulated cell proliferation in the VP, but not in the DLP. EGCG increased apoptotic indices in the VP, but not in the DLP.

Of the effect that the three polyphenols had on all the sex steroid receptors and growth factor signaling proteins, genistein is most effective in down-regulating proteins associated with cell proliferation (AR, IGF-signaling, EGFR and the activated ERKs). Genistein also decreased the ratio of cell proliferation to apoptosis. Resveratrol down-regulated IGF-1 and phospho-ERK-1 in the DLP and IGF-1R and the phospho-ERKs in the VP, but it resulted in elevated AR, and EGFR in the DLP. Nevertheless it did suppress poorly differentiated carcinoma development. What is of great interest is that EGCG suppressed H-PIN and down-regulated the IGF-1 signaling pathway and the phospho-ERKs in the VP at 12 weeks of age, but did not suppress PDC. Perhaps the elevated AR and EGFR in the DLP coupled with down-regulation of the IGF-signaling pathway is the key to determining the potential of these polyphenols to regulate prostate cancer development. Combination genistein and resveratrol resulted in down-regulation of IGF-1 and IGF-1R in the DLP even though the combination resulted in increased AR protein level. Hence, the constant is the down-regulation of the IGF-signaling pathway.

Another interesting finding is that the IGF-1 signaling proteins were differentially regulated by each polyphenol in the DLP and VP, leading us to conclude that in mice both lobes should be studied. It appears that MDC and WDC start in the DLP. However, PDC lesions start earlier in the VP than the DLP to result in the VP becoming the dominant tissue in the development of PDC. After PDC is established in the VP, cross talk may result in PDC development in the DLP.

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3) Wechter WJ, Leipold DD, Murray ED, Jr., Quiggle D, McCracken JD, Barrios RS, Greenberg NM. E-7869 (R-flurbiprofen) inhibits progression of prostate cancer in the TRAMP mouse. Cancer Research. 60(8):2203-2208, 2000.

Appendices

One Table
Eight Figures

Table 1. Histologic Analysis of Prostate Tumors from TRAMP Mice Fed Combination Genistein and Resveratrol in the Diet

Description	NT	L-PIN	H-PIN	WDC	MDC	PDC
			Dorsolateral Prostate			
Controls (27)	2%	5%	80%	4%	5%	1%
L-G+R (26)	0%	3%	68%	10%	11%	5%
H-G+R (23)	0%	9%	76%	7%	8%	0%
			Ventral Prostate			
Controls (25)	11%	18%	64%	0%	0%	15%
L-G+R (25)	4%	18%	70%	0%	0%	12%
H-G+R (22)	0%	45%	50%	2%	0%	3%
			Average for Dorsolateral + Ventral Prostate			
Controls	6.5%	10.5%	72.0%	2.0%	2.5%	8.0%
L-G+R	2.0%	10.5%	69.0%	5.0%	5.5%	8.5%
H-G+R	0%	27.0%	63.0%	4.5%	4.0%	1.5%

Genistein and resveratrol were provided in the diet at 250 mg/kg and 625 mg/kg AIN-76A diet (L-G+R) or 125 mg/kg and 313 mg/kg AIN-76A (L-G+R) or AIN-76A diet only (Controls) to the lactating dams and the offspring from parturition until the offspring were 30 weeks old. The numbers in parenthesis represent number of mice prostates evaluated. Normal tissue: NT; Low Prostatic Intraepithelial Neoplasia: L-PIN; High Prostatic Intraepithelial Neoplasia: H-PIN; Well Differentiated Carcinoma: WDC; Moderately Differentiated Carcinoma: MDC; Poorly Differentiated Carcinoma: PDC.

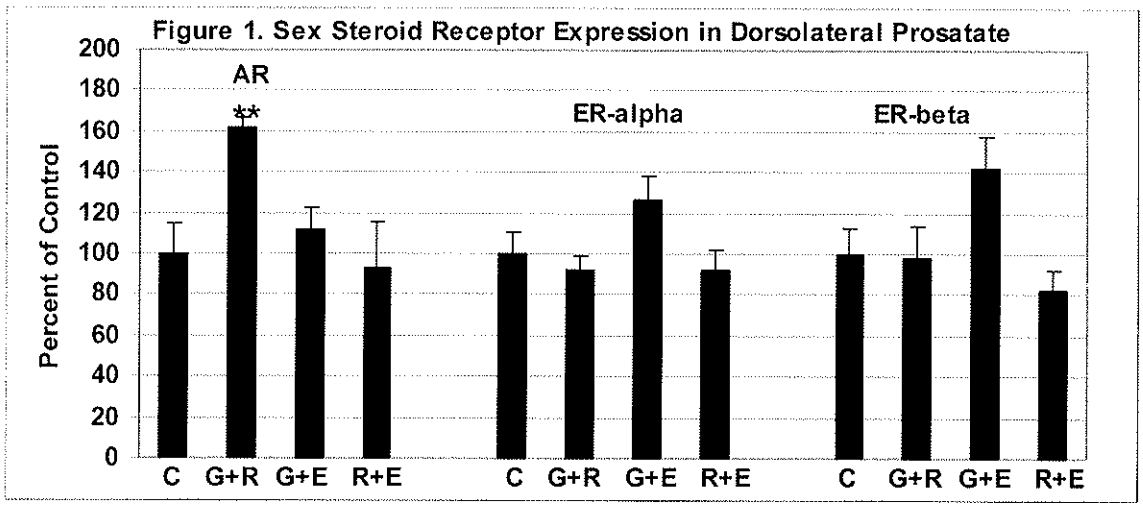
Figure 1. Sex Steroid Receptor Expression in Dorsolateral Prosatate
Percent of Control
200
180
160
140
120
100
80
60
40
20
0
AR
**
ER-alpha
ER-beta
C G+R G+E R+E
C G+R G+E R+E
C G+R G+E R+E

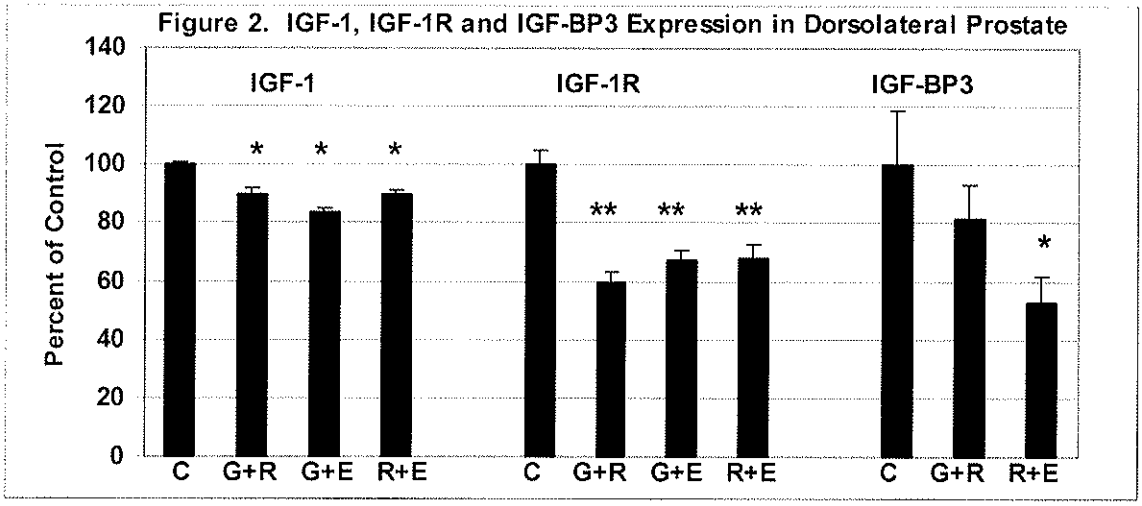
Figure 2. IGF-1, IGF-1R and IGF-BP3 Expression in Dorsolateral Prostate
Percent of Control
140
120
100
80
60
40
20
0
IGF-1
IGF-1R
IGF-BP3
* * *
** ** **
*
C G+R G+E R+E
C G+R G+E R+E
C G+R R+E

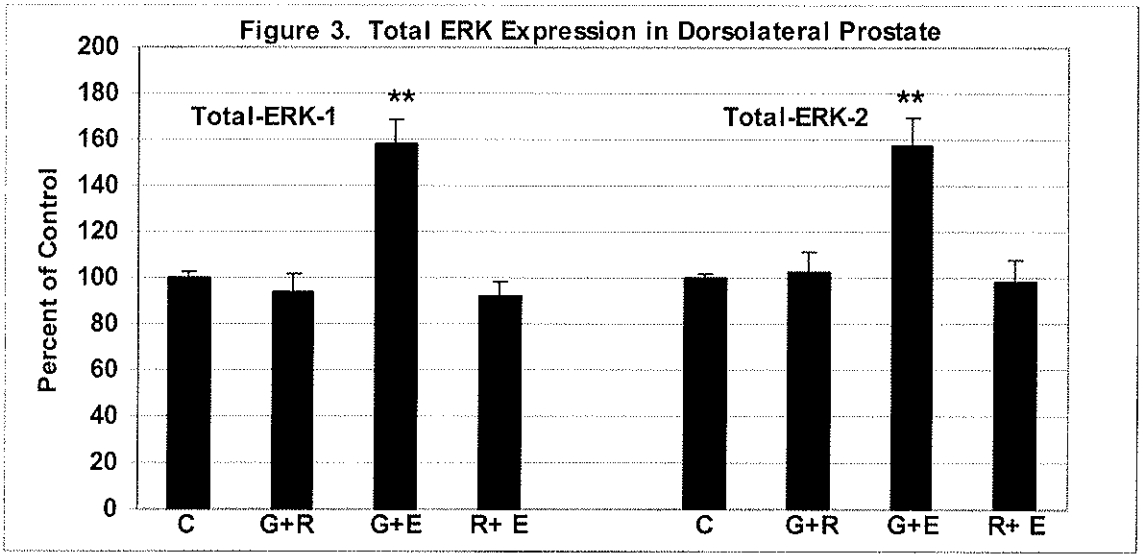
Figure 3. Total ERK Expression in Dorsolateral Prostate
Percent of Control
200
180
160
140
120
100
80
60
40
20
0
Total-ERK-1
**
Total-ERK-2
**
C G+R G+E R+ E
C G+R G+E R+ E

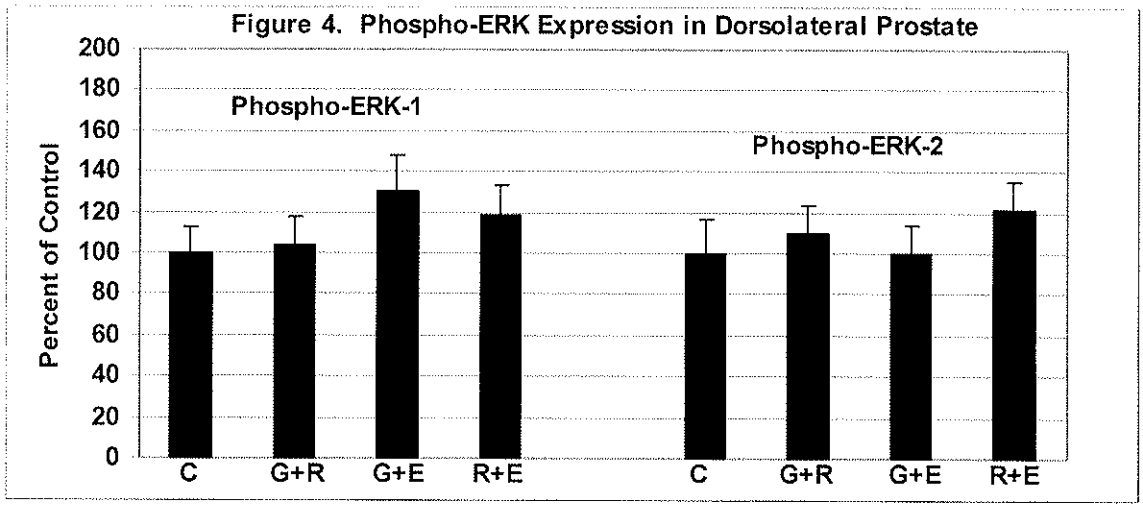
Figure 4. Phospho-ERK Expression in Dorsolateral Prostate
Percent of Control
200
180
160
140
120
100
80
60
40
20
0
Phospho-ERK-1
Phospho-ERK-2
C
G+R
G+E
R+E
C
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G+E
R+E

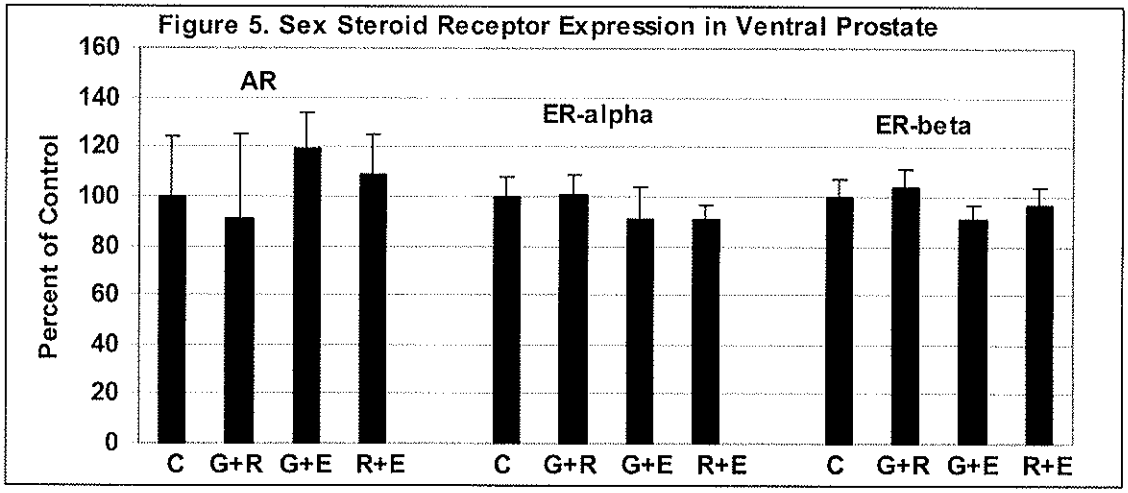
Figure 5. Sex Steroid Receptor Expression in Ventral Prostate
Percent of Control
160
140
120
100
80
60
40
20
0
AR
ER-alpha
ER-beta
C
G+R
G+E
R+E
C
G+R
G+E
R+E
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G+E
R+E

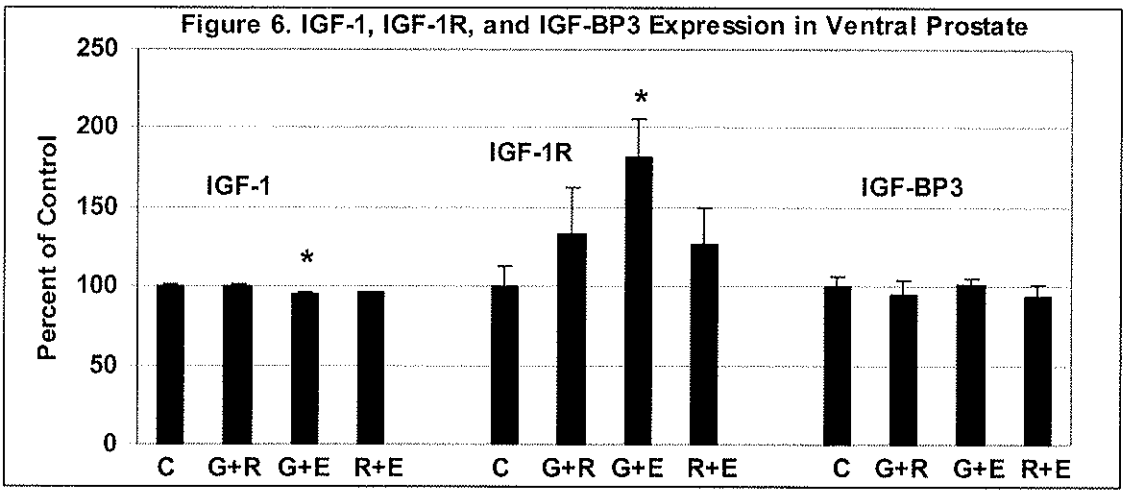
Figure 6. IGF-1, IGF-1R, and IGF-BP3 Expression in Ventral Prostate
Percent of Control
250
200
150
100
50
0
IGF-1
IGF-1R
IGF-BP3
*
*
C
G+R
G+E
R+E
C
G+R
G+E
R+E
C
G+R
G+E
R+E

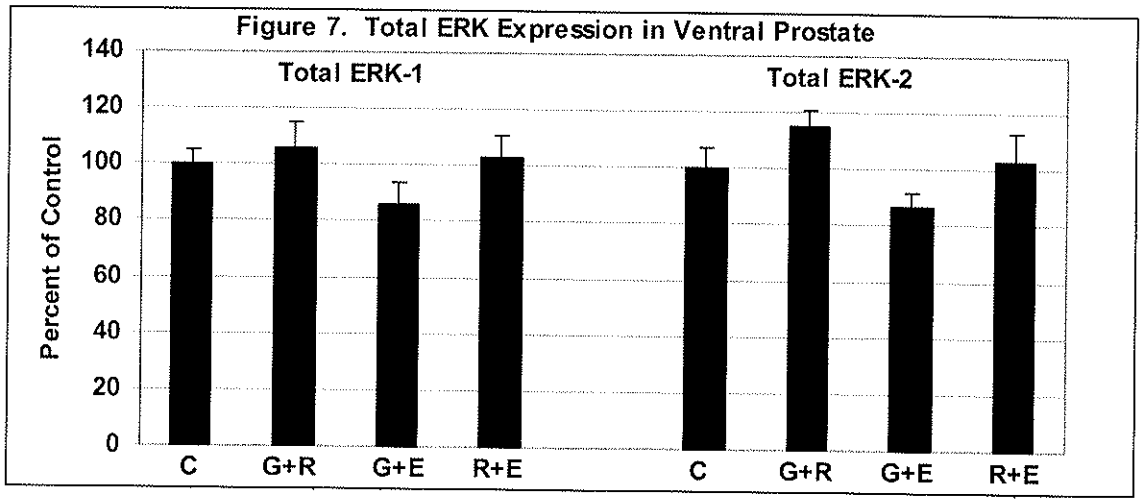
Figure 7. Total ERK Expression in Ventral Prostate
Total ERK-1
Total ERK-2
Percent of Control
140
120
100
80
60
40
20
0
C
G+R
G+E
R+E
C
G+R
G+E
R+E

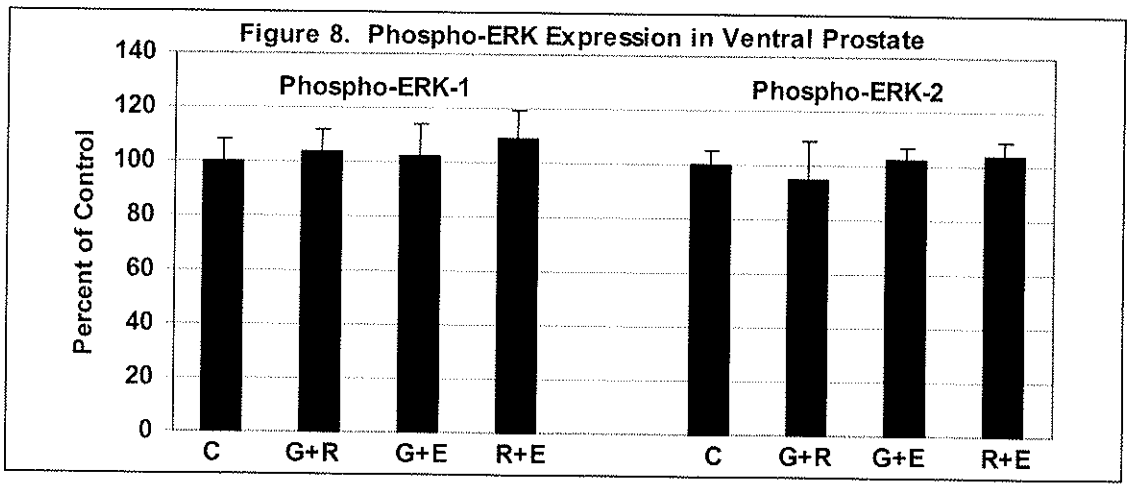
Figure 8. Phospho-ERK Expression in Ventral Prostate
Phospho-ERK-1
Phospho-ERK-2
Percent of Control
140
120
100
80
60
40
20
0
C
G+R
G+E
R+E
C
G+R
G+E
R+E